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Pressure Effects on Mammalian Central Nervous System

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INTRODUCTION

Pressure represents a fundamental thermodynamic state variable and, as such, changes induced by pressure reflect alterations in reaction rates or equilibria. High pressure (HP) is expected to universally affect molecules such as membrane phospholipids and proteins such as enzymes, transporters, ionic channels, cytoskeleton, etc. (for review see Daniels and Grossman, 2003, and dedicated chapters in this book). Therefore, molecular 'resistance' to pressure is one of the most important factors determining animals' adaptation to deep-sea dwelling as well as to deep diving. Recent studies, however, suggest that pressure effects are not uniform and may be selective. Pressure is expected to have slow reaction when increased volume is associated with the activated state of the molecule or the product of the reaction. It is expected to facilitate the process if the opposite is occurring. Thus, the consequent physiological effects are complex and dependent on many 'pressure sensitive' molecules in various cells. One of the most striking effects of pressure is on the nervous system. Exposure to HP is associated with the development of the high pressure neurological syndrome (HPNS) in humans and animals. HPNS signs and symptoms include malfunction of the autonomous nervous system, reduction of cognitive functions, decreased motor coordination, sleep disorders, and EEG changes. At greater pressures (deeper diving), serious signs such as tremors, convulsions and seizures leading to death may occur (see Chapter 20 in this book).

Therefore, in the present chapter, we shall review mainly HP effects in the physiological range of 0.1–10.1 MPa (1–100 ATA) on mammalian central

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nervous system (CNS). We shall attempt to reveal the associated cellular and circuit mechanisms in isolated CNS spinal cord and medulla, brain slices from various regions of the brain, and CNS synaptosomes. In some cases, we will refer to key data obtained from invertebrate preparations by using experimental methods that can not be implemented in CNS experiments at pressure.

NEURONAL MEMBRANE PROPERTIES

The effects of pressure on individual ion channels (see Chapter 7 in this book) can account for many of the pressure effects on neuronal activity described in this section. Several earlier review articles deal with pressure effects on passive and active properties of excitable cells: Wann and Macdonald (1980); Macdonald (1982); Halsey (1982); Kendig et al. (1993); Hogan and Besch (1993); and Daniels and Grossman (2003).

In order to measure the electrical properties of neurons such as membrane resting potential (V_{RP}), membrane resistance (R_m) that can be calculated from the experimentally measured input resistance (R_{in}), and action potential (AP), intracellular recordings must be made. Due to the technical difficulties involved, these data are limited in invertebrate preparations and rare in mammalian CNS. Early reports indicated no change of V_{RP} and small increase in R_{in} in squid giant axon (Spyropoulos, 1957a), while lobster axon V_{RP} was depolarized with some increase of R_{in} (Grossman and Kendig, 1984), probably due to the pressure depression of electrogenic Na^+–K^+ ATPase activity. In molluscan neurons depolarization was also observed, but accompanied by a decrease in R_{in} (Wann et al., 1979).

Only few studies have reported pressure effects on vertebrate neurons recorded intracellularly. There were no significant differences between mean values obtained for V_{RP} and R_{in} for two populations of rat CA1 hippocampal pyramidal neurons at atmospheric pressure and pressures up to 10 MPa (Southan and Wann, 1996). In contrast, Dean and Mulkey (2000) have reported that 38% of neurons sampled in the rat medulla solitary complex reversibly responded upon helium compression to as low as 0.3 MPa with 1–3 mV depolarization and 16% increase of R_{in}. This is probably an exceptionally pressure sensitive cell population, since the rest of the cells were not affected.

It is probable that pressure effect on mammalian CNS neurons electrical properties will differ from cell to cell due to differences in membrane channel populations, and the extent to which V_{RP} depends on the activity of Na^+–K^+ electrogenic pump. A small depolarization may increase excitability by bringing a cell closer to the threshold for impulse initiation, but may partially inactivate sodium channels with its consequent effects on AP.

ACTION POTENTIALS

Single response

Alteration of channel kinetics by pressure will produce changes in a single AP shape, amplitude, duration, and conduction velocity. Remarkably, early investigation of isolated myelinated frog nerve (Grundfest and Cattell, 1935; Grundfest, 1936; Spyropoulos, 1957a, b) at hydrostatic pressure revealed slower conduction velocity, an increase in duration of the extracellularly recorded electrical signal, and a lengthening of the refractory period. Intracellular recordings (which are not feasible in CNS axons) in squid giant axon (Shrivastav et al., 1981), lobster axons (Grossman and Kendig, 1984; Grossman et al., 1986) and molluscan cell bodies (Wann et al., 1979) revealed that various axon populations might respond differently to pressure. Yet, in most preparations, pressure (at steady state) slowed the kinetics, slightly reduced the amplitude and decreased the conduction velocity of the AP. These alterations mirror accompanying changes in peak sodium current amplitude. These studies also likened the effect of pressure to reduced temperature. These findings were corroborated by later studies in several lobster neuromuscular synapses in which extracellular macropatch current recordings were performed from the presynaptic axon terminals (Grossman et al., 1991a; Golan et al., 1994, 1995, 1996). The small diameter terminals seem to be more sensitive to pressure since amplitude reduction in many of the synaptic sites could reach 30%.

For many years the information on neuronal activity of mammalian brain was obtained by extracellular field potentials recorded either *in vivo* from rodents' brain or *in vitro* from isolated rat brain structures or slices. This type of field potential recording reflects the activity of populations of neurons, axons, and synapses. Studies by Fagni et al. (1987a, b), Zinebi et al. (1988a, b), Talpalar and Grossman (1995, 2003, 2004), and Southan and Wann (1996) have demonstrated that orthodromic population spike that is evoked by synaptic mechanism may be reduced by various degrees. However, antidromic population spike that is induced by direct stimulation of the axons is usually only slightly changed. Etzion and Grossman (1999) succeeded for the first time in recording at pressure single Purkinge cell activity in guinea pig cerebellar brain slices. In these cells, the somatic Na^+ AP current was not affected by pressure, the dendritic Ca^{2+} spike current amplitude was only slightly reduced, but its kinetics was slowed by 15%. Another study (Etzion and Grossman, 2000) has shown that the parallel fibers presynaptic volley, that reflects the AP (mostly Na^+ current) of a population of axonal terminals, is reduced by 25% at 10.1 MPa. In contrast, Mor and Grossman (2006) have reported only a tendency for a slight reduction in amplitude of the presynaptic volley of the Schaefer collaterals

in the hippocampal CA1 area, although without statistical significance. Intracellular recordings from mammalian neurons revealed that neither low hyperbaric pressure (Dean and Mulkey, 2000) nor high pressure (Southan and Wann, 1996) significantly affect the kinetics of Na^+ AP. However, pressure did reduce the K^+-conductance dependent after hyperpolarization (AHP) of the spikes. This modulation should affect the firing pattern of the cells (see below).

Finally, few studies on nerve conduction in humans have been performed during simulated dives to 450m (Grapperon et al., 1988) and 360m (Todnem et al., 1989) in dry pressure chambers. Both studies reported reduced conduction velocity for fast sensory axons and increased delay at the distal part of the motor nerves.

Patterns of action potential activity

Several patterns of AP activity have been tested under pressure conditions: branch point failure, spontaneous repetitive firing, and rhythmic (pacemaker) activity. The lobster axon used in the aforementioned studies has the interesting property of failing at its branch point during short trains of action potentials at physiological high frequencies (Grossman et al., 1979a, b). This low safety factor conduction is probably an important feature of distal dendrites and terminal brunching. Pressure at steady state impaired conduction through the branch point (Grossman and Kendig, 1986, 1987).

Mammalian CNS axons normally do not fire repetitively at pressure. However, a small portion of axons in crustacean (Kendig et al., 1978), squid (Spyropoulos, 1957a) and vertebrate peripheral nerve (Grundfest, 1936; Grundfest and Cattell, 1935) responded with spontaneous impulse generation or repetitive activity in response to single stimulus at 10–40 MPa pressures range.

Many CNS neurons exhibit variable oscillatory or pacemaker activity. Autorhythmic respiratory-drive frequency, recorded extracellularly in the cervical spinal ventral roots in isolated newborn rat brainstem-spinal cord preparation, was reduced at 10.1 MPa (Tarasiuk and Grossman, 1991). In contrast, in this preparation, pressure also induced an opposite response: increased spontaneous, stimulus-induced, or respiratory drive associated tremor-like activity in lumbar and cervical ventral roots motor neurons (Tarasiuk and Grossman, 1990). Moreover, Dean and Mulkey (2000) have shown that highly barosensitive neurons in the rat medulla increased their sustained firing rate at 0.3 MPa. In guinea pig cerebellar Purkinje cells (Etzion and Grossman, 1999) HP effects are complex. Pressure differentially altered the pattern of dendritic Ca^{2+} APs activity, but did not affect the spontaneous somatic Na^+ spikes. Pressure prolonged the active period of spike firing by 52%, but had no effect on the quiescent period of the cycle.

Furthermore, within the active period, pressure reduced the mean firing rate by 65% and suppressed the development of typical firing of spike doublets. These changes are probably the result of pressure modulation of voltage and $[Ca^{2+}]_i$-dependent K^+ currents that underlie spike AHP and pacemakers activity. The changes indicate reduced excitability of the dendrites. In studies of rat hippocampal dentate gyrus axons (Talpalar and Grossman, 2003, 2004) HP did not affect the frequency response of the axons to direct stimulation. In contrast, the recent study of hippocampal CA1 pyramidal neurons (Mor and Grossman, 2007a) showed reduced capability of the neurons to produced Na^+ population spikes following repetitive synaptic activation under conditions of NMDAR activity alone.

In summary, pressure usually slightly increases R_{in} and depolarizes neuronal membrane. Notable changes in V_{RP} are observed in those neurons in which electrogenic Na^+-K^+ ATPase activity contributes considerably to the V_{RP}. Pressure slightly reduces the amplitude of both Na^+ and Ca^{2+} APs in axons; nonetheless, the effect is quite prominent in axonal terminals. The most predominant pressure effect is the reduced kinetics of the AP which consequently decreases conduction velocity and frequency response. Pressure modulation of various K^+ channel types, that usually underlie spike AHP and pacemaker activity, probably determine the tendency to fire either spontaneous or evoked trains of APs at a particular frequency or specific pattern. These modifications, while not 'dramatic', will probably disturb normal CNS synaptic transmission (see below), impair time and event detection, and modify neuronal and circuit rhythmic activity.

EXCITATORY SYNAPTIC ACTIVITY

It is conceivable that both HPNS motor hyperexcitability and cognitive impairments are the consequence of disturbance in network synaptic activity. We will describe only pressure effects on chemical synapses since electrical synapses have not been studied at pressure. The normal CNS activity is based on well-coordinated and well-balanced interactions between excitatory and inhibitory (see below) synaptic pathways. The most common excitatory transmitter in the CNS is glutamate. However, several different postsynaptic receptors are mediating glutamate transmission: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, quisqalate, and N-methyl-D-aspartate (NMDA) sensitive receptors. They all gate cation channel for Na^+, K^+, and Ca^{2+}, but NMDA receptor (NMDAR) has relatively much higher conductance of Ca^{2+} (about 11% of the current is carried by Ca^{2+}).

In the 1980s, with the limited cellular methods for CNS studies, a large body of work was carried out on the behavior of animals, testing their sensitivity to HP (threshold for tremor and seizure, HPNS) using pharmacological antagonists or sometimes agonists of the specific receptors.

Selective and non-competitive antagonists at non-NMDARs show both species and effect-specific actions. While some types have no effect against pressure in the rat (Wardley-Smith and Meldrum, 1984), others may be effective against tremor but not against seizures (Wardley-Smith et al., 1987), or effective against both symptoms (Pearce et al., 1994).

Initial experiments using the NMDAR antagonists (Meldrum et al., 1983) or competitive antagonists (Wardley-Smith and Meldrum ,1984; Wardley-Smith et al., 1987; Pearce et al., 1991, 1993) demonstrated various degrees of protection against hyperexcitability, tremors, and seizures caused by HP. Non-competitive NMDAR antagonists exhibited species and drug dependent effects. For example, ketamine is effective against all aspects of HPNS in the rat (Angel et al., 1984), while MK801 has no effect in the rat (Wardley-Smith and Wann, 1989), but gives some protection in the baboon (Pearce et al., 1990). It is conceivable that the differences between species, as well as in drug potency, may arise from different NMDAR subtypes existing in various animals (for details see Daniels and Grossman, 2003, and Chapter 7 in this book).

Glutamatergic AMPA synapses

Single response

All AMPA receptors (AMPAR) single field excitatory post synaptic potentials (fEPSP) tested so far in mammalian brain, as well as those at crustacean neuromuscular junction (see rev. Daniels and Grossman, 2003), are depressed and their kinetics is slowed at HP. This was demonstrated in brain slices preparations of rat hippocampal Shaefer collaterals synapse on CA1 pyramidal cells (Fagni et al., 1987a), guinea pig cerebellar parallel fibers (PF, Etzion and Grossman, 2000) and climbing fibers (CF, Etzion et al., 2008) synapses on Purkinje cells, and rat dentate gyrus medial perforant path synapse on granule cells (PP, Talpalar and Grossman, 2003, 2004). The most significant HP effects are the decrease of fEPSP amplitude, reduction of its initial slope, prolongation of decay time, and increase of synaptic delay. Although fEPSPs were depressed, the ability of the neurons to produce population spikes either remained stable (Talpalar and Grossman, 2004) or even enhanced (Fagni et al., 1987a). These indicate an increased gain of the system despite the severe 50% depression of the synaptic input to the cells. A similar effect was observed in isolated newborn rat medulla- spinal cord preparation. Synaptic responses were not studied directly in these models. However, in addition to increased excitability of the motorneurons (Angel and Halsey, 1987; Tarasiuk and Grossman, 1990), HP of 10.1 MPa slowed the kinetics but did not affect the amplitude of spinal monosynaptic reflex and even increased by 30% various polysynaptic reflexes (Tarasiuk and

Grossman, 1989; Tarasiuk et al., 1992). The reflexes' electrical responses recorded from cut ends of spinal ventral roots reflect a compound action potential (not synaptic potentials) elicited in the motorneurons pool and / or population of medullar neurons as a result of reduced synaptic inputs. Thus, they also demonstrated increased gain of the system. Possible explanations for this unique phenomenon could be the involvement of NMDAR, reduced circuit inhibition, and possible boosting of the signal along the neuron dendrites by Ca^{2+} channels activity (see below).

Frequency modulation

Synaptic plasticity as a function of activity is a fundamental feature of chemical synapses. HP alters the short term frequency response of the synapses. Pressure affects even the simple paired pulse modulation. HP reduces paired pulse depression (PPD) normally observed at short (< 20 ms) inter stimulus intervals (ISI), but increases paired pulse facilitation (PPF) normally observed at longer ISIs, in the dentate gyrus PP synapses (Talpalar and Grossman, 2003, 2004,). A qualitatively similar effect was observed in cerebellar PF synapses, but surprisingly, HP did not affect a general PPD in the cerebellar CF, a saturated-release type synapse (Etzion et al., 2008). In contrast to invertebrate synapses (see rev. Daniels and Grossman, 2003), frequency-dependent synaptic response for trains (5–10 stimuli) of AMPAR fEPSPs are bimodally modulated by HP. Normally, the second fEPSP is facilitated and the rest of the responses in the train are progressively depressed. HP enhanced this frequency dependent depression (FDD) at 50 Hz, while at 20 Hz the FDD was maintained. However, when the synaptic gain of the system (as above) was tested by evoking population spikes in the cells following a paired pulse protocol and short trains of stimuli, the picture changed. Despite the depression of single fEPSP, and in accordance with increased PPF, the gain of the system remained unchanged at HP for paired pulses. Population spike generation during 25 Hz train stimulation was conserved at pressure. However, at 50 Hz HP induced additional spikes per stimulus, indicating disruption of the regular low-pass filter properties of this dentate gyrus network (Talpalar and Grossman, 2004, 2005).

Presynaptic mechanism

HP suppression of evoked synaptic AMPA transmission is effected mainly by presynaptic mechanisms. Fagni and his colleagues (Fagni et al., 1987a, b; Zinebi et al., 1988a, 1990) examined in the hippocampal CA1 region the efficacy of various excitatory neurotransmitters that were perfused in the bath, in reducing fEPSP and population AP under normobaric and

hyperbaric conditions. HP had no significant effect on the capacity of various agonists of the non-NMDA glutamate-like receptors such as of L-glutamate, quisqualate, L-aspartate, kainate, and D-homocysteate, in modulating the responses. Further support to the notion that AMPAR seems to be insensitive to pressure comes from direct tests of the receptors expressed in oocytes (see Chapter 7 in this book). The best way to demonstrate the physiological presynaptic effect is a detailed statistical quantal analysis of the release process. Such analysis at HP has been performed only in crustacean neuromuscular glutamatergic synapses (Golan et al., 1994, 1995, 1996). However, indirect studies suggest that the presynaptic mechanisms affected by HP in the CNS are similar to those of crustaceans. These include decreased quantal content (m), small variable change in probability of release (p), and reduction in the number of the release sites (n). Reduction in n is interpreted in the CNS synapses as a diminution of the readily releasable pool of vesicles (Talpalar and Grossman, unpublished data). All these parameter changes attest to presynaptic site of action. In contrast, HP has no effect on the quantal size (q), namely the unitary response to transmitter released from a single vesicle. This indicates lack of effect on the post synaptic receptor's response.

Previous experiments on synaptosomes prepared from rodents CNS tissue also support the notion of presynaptic effects of pressure. Synapto-some is a sealed broken nerve terminal, presumably containing the whole transmitter release apparatus, but without a postsynaptic component. The synaptosomes were preloaded with radioactive-labeled neurotransmitter, washed, and then the Ca^{2+}-dependent release caused by steady depolariza-tion of the membrane (effected by increased $[K^+]_o$) was monitored. HP (6.9 MPa) suppressed release of the glutamate, but did not affect release of similar less abundant aspartate (Gilman et al., 1986a). The major pressure effect was slowing of the release during the first minute or two after stimula-tion, and in some cases, a moderate reduction in the maximal release. The technique used in these experiments can only measure transmitter release on a time scale of minutes or few seconds, which is considerably slower than the actual milliseconds time course of normal synaptic trans-mission; however, within this limitation, they demonstrate a large pre-synaptic effect of ambient pressure.

Exocytosis *per se*, i.e. the fusion of vesicles with the plasma membrane of the nerve terminals, could play a role in the pressure sensitivity of fast synaptic transmission. This question was addressed by Heinemann et al. (1987a) who studied degranulation (secretion) of bovine adrenal cells and peritoneal rat mast cells. Cells were stimulated by dialysis of 1 μM free calcium or 40 μM GTP γ-S (an intracellular second messenger) via a patch pipette, and the change in cells membrane capacity as an indicator for vesicles fusion was monitored by voltage clamp techniques. Even modest pressures caused an appreci-able decrease in the rate of exocytosis in these

non-neuronal cells. The sensitivity to pressure is 4–5 times greater than that of synaptic transmission (estimated by calculation of activation volumes). Since Ca^{2+} influx is bypassed by this method, the results show that pressure interferes also with the exocytosis itself. However, the high sensitivity of the slow exocytosis may not directly reflect on the fast synaptic signaling, but since such a mechanism is ubiquitous in neurohormonal and neuromodulator secretion, it is postulated that pressure would have some long term modulatory effects on brain function. Yet some of the exocytosis intermediate steps may determine the frequency response of the fast synapses (as above).

Ca^{2+} and presynaptic release

Synaptic release is a multi-step mechanism. The first crucial stage is increased Ca^{2+} influx and elevation of cytosolic Ca^{2+} concentration at the nerve terminal following membrane depolarization by the invading AP. The subsequent step is fusion of docked vesicles with the terminal plasma membrane which ends in evoked release. In parallel, Ca^{2+} entry activates mobilization processes that detach vesicles from their reserve pool, translocate them to the readily releasable pool and prime them at the releasing sites. Many previous experiments on invertebrate preparations, including use of theoretical models (Grossman and Kendig, 1990) and quantal analysis under reduced $[Ca^{2+}]_o$ conditions (Golan et al., 1994, 1995, 1996) revealed striking similarity to HP in parameters change. This strongly indicates that reduced Ca^{2+} entry into the presynaptic terminals during the action potential is the major cause for depressed evoked synaptic transmission. While reduced $[Ca^{2+}]_o$ mimicked HP depression, increased $[Ca^{2+}]_o$ could antagonize HP depression in these experiments, both supporting this model. Indeed, HP also impeded depolarization–induced influx of Ca^{2+} into CNS guinea pig cerebrocortical synaptosomes through unidentified channels (Gilman et al., 1986b). In contrast, HP did not change the Ca^{2+} flux through artificial Ca^{2+} ionophore (A 23187) inserted into these synaptosomes (Gilman et al., 1991). Furthermore, HP effects on single and paired responses in the CNS are consistent with the above mechanism. However, in vertebrate (Grossman et al., 1991b) and especially in mammalian CNS the release processes are more complicated, since the terminals usually contain more than one type of Ca^{2+} channels (Takahashi and Momiyama, 1993) that may be differentially affected by HP (Aviner and Grossman, 2008, and unpublished data), and the frequency response may be nonlinear and biphasic (as mentioned above). HP affects the presynaptic terminals probably by impeding Ca^{2+} influx through a combination of voltage gated Ca^{2+} channels (Talpalar and Grossman, 2003). However, the most pressure sensitive Ca^{2+} channel is the N-type, as demonstrated in frog neuromuscular

terminals (Grossman et al., 1991b), indicated for lobster neuromuscular synapse (Grossman et al., 1991a) and suggested for cerebellar CF synapse (Etzion and Grossman, 2000).

Experiments on modification of $[Ca^{2+}]_o$ in CNS synapses were similar to invertebrate results with respect to single responses, but varied by frequency response of different CNS synapses. In the dentate gyrus PP synapse, reduced $[Ca^{2+}]_o$ to half of its normal value (1mM) increased PPF to a greater extent than HP (10.1 MPa) at normal $[Ca^{2+}]_o$. This reduced $[Ca^{2+}]_o$ unlike pressure turned FDD into a frequency dependent potentiation (Talpalar and Grossman 2003). Increased $[Ca^{2+}]_o$ (4–6 mM) at atmospheric pressure, saturated single fEPSP and increased FDD in response to short trains at 50 Hz. Increasing $[Ca^{2+}]_o$ to 4 mM at HP saturated synaptic release at a subnormal level (only 20% recovery of a single fEPSP), but enhanced FDD with an efficacy similar to that at atmospheric pressure. Analysis of the normalized fractions of release during the train and the consequent 'probability' of release revealed that HP may depress synaptic frequency response by reducing the size of the readily releasable pool of synaptic vesicles (Talpalar and Grossman unpublished data). In the cerebellar PF synapse, low $[Ca^{2+}]_o$, as well as N-type and N+P / Q –type Ca^{2+} channel blockers that reduce presynaptic Ca^{2+} entry, inhibit the single synaptic response to a similar extent as HP (Etzion and Grossman, 2000). These treatments significantly increased the PPF (118–125 %), but only at 20 ms ISI, whereas pressure effect was for a longer ISIs as well (Etzion et al., 2008). In the cerebellar CF synapse, low $[Ca^{2+}]_o$ (0.5 –1 mM) mimicked HP effect on the single response, but reduced also the PPD, while HP did not affect PPD. Doubling the level of $[Ca^{2+}]_o$ (4 mM) could not antagonize the depression of the response at HP. Further increase of $[Ca^{2+}]_o$ to 6 mM could slightly antagonize the effect of pressure on the amplitude of the first fEPSP, but it was accompanied by increased PPD.

In summary, HP depresses the response of all glutamatergic excitatory AMPAR-dependent synapses tested so far. The depression is primarily of presynaptic origin, i.e. reduced released quanta of transmitter and increased synaptic delay. Evidence on the role of $[Ca^{2+}]_o$ in single fEPSPs and very few Ca^{2+} current measurements strongly indicate that pressure impedes Ca^{2+} entry into the presynaptic terminals. Recent studies in the cerebellum suggest that pressure effects on various voltage gated Ca^{2+} channels may be selective; the prevalence of N-type in the terminals seems to correlate with pressure vulnerability. However, during repetitive activation such as paired pulse and frequency modulations, HP probably interferes with additional mechanisms other than Ca^{2+} entry that are involved with the cascade of synaptic release. Yet, frequency potentiation at pressure may partially compensate for the depression of single response. The extent at which HP will affect a particular synapse is dependent on its set-point release operation

(with regard to saturation), the type of Ca^{2+} channels associated with the release, and its frequency response characteristics.

Exocytosis itself may not play a major role in fast transmission (during which vesicles and terminal membranes fusion does occur), however it certainly is depressed in slow secretion of neuromodulators and neurohormons.

It is conceivable that the large depression in excitatory synaptic activity will cause functional difficulties for any neuronal circuit. However, it can not explain the hyperexcitability and epileptogenesis observed at HPNS.

Glutamatergic NMDA synapse

In early studies in brain slices, Fagni and his colleagues (Fagni et al., 1987a, b; Zinebi et al., 1988a, 1990) examined in the hippocampal CA1 region the efficacy of various excitatory neurotransmitters and agonists that were perfused in the bath, in reducing fEPSP and population AP under at HP (as above). Whereas HP had no significant effect on the non-NMDA responses, it greatly increased NMDA and L-homocysteate efficacy. This indicates that pressure increases, rather than decreases, the activity of the latter two amino acids receptors. This intriguing result was confirmed by direct measurements of the current of NMDAR from rat cerebellum expressed in *Xenopus laevis* oocytes (see Chapter 7 in this volume). Recently, Mor and Grossman (2008) have shown that this increase may be selective for specific combination of the NMDAR NR1-NR2 subunits. Mor and Grossman (2006, 2007a) have also succeeded in isolating the responses of NMDAR in Shaefer collaterals synapses onto the pyramidal cells in rat hippocampal CA1 brain slices by blocking AMPA and $GABA_A$ neurotransmission and removing Mg^{2+} from the physiological solution (in order to enable receptor opening without prior depolarization of the AMPAR). These are the only available studies on NMDAR fEPSPs under HP conditions, the data of which are described below.

Since NMDARs are usually co-localized postsynaptically with AMPARs in neuronal synapses, they are exposed to glutamate released by the same presynaptic mechanism. Therefore a special paragraph on release is absent from this NMDAR description.

Single response

The single fEPSP of NMDAR is enhanced by HP and its kinetics is greatly slowed down. HP (10.1 MPa) increased synaptic delay by 16% and maximal initial slope by of single fEPSP by 80% and its time integral by 250%! This implies a great increase in the total charge influx which is carried by Na^+ and a significant portion of Ca^{2+}. Such abnormal elevation of postsynaptic

(dendritic) influx of Ca^{2+} may trigger known cytotoxic processes that could facilitate neuronal death (Lynch and Guttmann, 2002).

Additional findings demonstrate that HP greatly reduces the sensitivity of the fEPSP to $[Mg^{2+}]_{o,}$ its natural ion-channel blocker, and to 2-amino-5-phosphonovaleric acid (AP-5), a pharmacological blocking agent (Mor and Grossman, 2007b). These important observations strongly indicate that HP causes conformational changes in the NMDAR.

Frequency modulation

HP decreased PPF (slope measurements) by 29% and 36% at 10–20 ms ISIs (50–100 Hz) and 40 ms ISIs (25 Hz) respectively. However, the results were not statistically significant due to increased variability of the second responses for an unknown reason. However, trend analysis revealed that PPF at high pressure was always smaller than the control values for each experiment. This is in accord with the increase in single response at high pressure. Frequency response was tested by trains of five fEPSPs at low frequency (25 Hz) and high frequency (50–100 Hz). At control pressure, for 25 Hz stimulation, the third, fourth, and fifth fEPSPs were depressed (FDD) following PPF of the second response. At HP, the first response was significantly augmented and PPF was reduced (as above), while the FDD was significantly enhanced. For 50–100 Hz trains, there was a similar behavior although HP did not affect the FDD. HP did not affect fEPSP train's time integral at 100 Hz, but it increased it by 96% at 25 Hz. This means again approximately two fold increase in Ca^{2+} influx into the postsynaptic dendritic tree. It is important to note that in contrast to the PP intact glutamatergic synapses (as above), in which the synaptic efficacy in producing population spikes was increased at pressure (gain increase), the isolated NMDAR response was maintained. In fact not only the delay to the first spike is reduced, but also the number and frequency of population spikes induced in each fEPSP and their total number during a train was significantly reduced. In summary, increasing evidence indicate that HP potentiates NMDAR response. However, this increase alone has a complex effect on the capability of the synapse (deprived of its AMPA component) to generate population spikes. Despite the increase of a single fEPSP, consecutive fEPSPs just maintain synaptic efficacy with no gain increase of the system. Under these conditions there is no indication for major increase of excitability that is observed in intact hippocampal synapses (see above). It seems reasonable that the full effect of pressure modulation of the NMDAR on neuronal excitability would be attained only when the fast AMPAR component of the synapse is intact. While these findings may suggest partial explanation for HPNS, the increase of fEPSP time integral which is associated with excessive influx of Ca^{2+}, may also provide possible molecular basis for

recent reports concerning long term deleterious health effects on professional divers associated with saturation dives.

INHIBITORY SYNAPTIC ACTIVITY

The examination of synaptic inhibition at HP is of obvious importance in order to understand the underlying mechanisms associated with pressure induced hyperexcitability (i.e. HPNS). As described above, AMPAR excitatory synaptic input is depressed and despite some PPF can not contribute to the hyperexcitability. NMDAR response is increased despite the depression of presynaptic release of glutamate, and therefore may partially compensate for the impaired AMPAR excitation. Inhibition is an integral part of any neural circuit. Pressure-induced reduction in inhibitory processes, concomitantly with some compensation of the excitatory mechanisms will disrupt the delicate balance between excitation and inhibition in the CNS and may ultimately cause hyperexcitability. Since transmitter release from the presynaptic terminals is believed to be similar for excitatory and inhibitory synapses, it will not be surprising that HP similarly depresses inhibition and excitation in model synapses. However, if an additional effect on receptor activity is exhibited (e.g. NMDAR), pressure effects could be more complicated.

Several ionic mechanisms may be involved in two major types of known functional inhibition. In the most common 'postsynaptic inhibition', the excitatory and the inhibitory axons are both synapsing on the dendritic or somatic membrane of the target neuron, which in turn integrates their EPSPs and inhibitory postsynapic potentials (IPSPs). In the 'presynaptic inhibition', the inhibitory axons synapse on the excitatory axons prior to their terminals on the target neuron, and thus may selectively reduce their input by shunting the AP. γ-amino-butyric acid (GABA) in the brain and glycine in the spinal cord are the most common CNS inhibitory neurotransmitters. Here the HP effects on the GABA type A receptor ($GABA_AR$) and glycine receptor (GlyR) will be described, both ionotropic receptors that gate Cl^- channel.

As mentioned above for the excitatory neurotransmission, in the 1980s an immense effort was invested in studying the role of the inhibitory neurotransmitters in HPNS as well by applying various related drugs (agonists and antagonists) to behaving animals (usually rats). Competitive antagonists of $GABA_AR$ (e.g. bicuculline) and GlyR (e.g. strychnine), as well as drugs that block the chloride channel associated with the receptors (e.g. picrotoxin) are convulsant in action. They all seem to mimic pressure effects and thus are reminiscence of HPNS (Bowser-Riley et al., 1988). The $GABA_AR$ also possesses a recognition site for benzodiazepines (e.g. diazepam) which increase the receptor response and thus are powerful sedatives and anticonvulsants (i.e. antiepileptic). The benzodiazepines provide some

protection against the effects of pressure (Halsey and Wardley-Smith, 1981), although at sedative doses. Prolonging the action of GABA by preventing its metabolism via inhibition of GABA transaminase (e.g. aminooxyacetic acid) or blocking its reuptake (e.g. 2, 4-diaminobutyric acid) provides some protection against pressure effects (Bichard and Little 1984). Increasing inhibition by administration of $GABA_AR$ agonist (e.g. muscimol) has no effect on the action of pressure on the whole animal. Aromatic propandiol compounds (e.g. mephenesin), which are known as muscle relaxants, are most effective against both pressure and strychnine induced convulsions, but not against GABA antagonists (Bowser-Riley, 1984; Bowser-Riley et al., 1989). In contrast, the aliphatic propandiol compounds (e.g. meprobamate) are effective anticonvulsants against GABA antagonists, but not against strychnine. It seems that the potency of these drugs against pressure effects was unrelated to their muscle relaxant efficacy. These partial lists of findings provide a compelling evidence to support a role for glycine and GABA mediated inhibitory neurotransmission in the etiology of HPNS.

GABAergic synapses

Direct measurement of GABAergic IPSP at HP was carried out only in lobster neuromuscular synapses (Grossman and Kendig, 1988). The muscle is innervated by a single glutamatergic excitatory and a single GABAergic inhibitory motorneuron. Single IPSPs were depressed at HP (10.1 MPa), concomitantly with 60% reduction in the functional postsynaptic inhibition (evoked by short train of stimuli to the inhibitor) of the EPSP, which is the same extent as in the excitatory transmission itself. In a different crustacean neuromuscular model Golan et al. (1994) have demonstrated that GABAergic presynaptic inhibition of the excitatory synaptic input is similarly depressed at HP. Using spinal cord synaptosomes experiments (see above) Gilman et al. (1987, 1989a) have demonstrated that GABA release at pressure is reduced and its kinetics is slowed, similarly to the glutamate release, supporting again the notion that the major effect of pressure is presynaptic. This is also corroborated by later findings indicating that $GABA_AR$ is insensitive to pressure (Shelton et al., 1996).

An attempt to indirectly evaluate the degree of GABAergic inhibition involvement in CNS HP effects was carried out by Zinebi et al. (1988a, b) in rat hippocampal CA1 region. Antidromic stimulation of the pyramidal cells' axons evoked recurrent inhibition, which prevented the generation of single-unit action potential evoked by stimulation of the SC synaptic input to these neurons. This inhibition became ineffective in an all-or-none fashion at HP. Similarly GABAergic feed forward inhibition onto these cells was also decreased. Another attempt to evaluate the contribution of inhibition mediated by $GABA_AR$ at HP was carried out recently in the PP synapse in

rat dentate gyrus brain slices (Talpalar and Grossman, 2004). Partial blockade of the $GABA_AR$ inhibition with low doze of bicuculline mimicked pressure effect, i.e. reduction of the frequency dependent attenuation of population spikes in the granular cells. Both HP and $GABA_AR$ blockade increased the number of population spike generated during a short train of stimuli at 50 Hz. Furthermore, under effective block of the $GABA_AR$ with higher doze of bicuculline, exposure to HP no longer increased excitability, but rather decreased it.

Functional inhibition of the MSR in a newborn rat's isolated spinal cord was also examined at HP (Schleifstein-Attias et al., 1994). MSR was recorded in hemisected spinal cord from severed end of lumbar ventral roots in response to stimulation of the corresponding dorsal root. Inhibition was evoked by prior stimulation of an adjacent dorsal root. By modulating the ISI between the two stimuli and the use of specific blockers, it was possible to demonstrate a complex time course of this inhibition. It is composed of an early phase (1–25 ms ISI) that is glycine dependent postsynaptic inhibition (see below), an intermediate phase (25–200 ms ISI) that is $GABA_AR$ dependent postsynaptic (and possibly also presynaptic) inhibition, and a late phase (200–1000 ms ISI) that may represent non-synaptic 'primary afferent depolarization' inhibition (Rudomin, 1990; Hackman and Davidoff, 1991). HP completely abolished the glycinergic inhibition, reduced the GABAergic inhibition by 68 %, and did not affect the late phase. In a different experiment a strong inhibition of the MSR was induced following 1–15 conditioning stimuli applied to the adjacent ventral root (Schleifstein-Attias and Grossman unpublished data). Using various ISIs and specific receptor blockers (as above), it was demonstrated that stimulation of the motoneurons axons evoked a short term recurrent inhibition (5–300 ms ISI) that was $GABA_AR$, glycine, and acetylcholine receptor dependent (see below) and also a long lasting inhibition (0.3–100s) that was probably serotonin dependent (see below). HP reduced the short term inhibition but had no effect on the long lasting inhibition. It is important to note that the pressure-induced suppression of the inhibition could be mimicked under normobaric conditions by reducing $[Ca^{2+}]_o$ by 50 % of the normal concentration. Raising $[Ca^{2+}]_o$ by 50 % could compensate for HP (10.1 MPa) effects.

In summary, HP depresses the $GABA_AR$ response of inhibitory synapses to the same extent as in the AMPAR-dependent synapses. Although strong direct evidence is missing, it seems conceivable that in the lack of HP effect on the $GABA_AR$, the depression is primarily of presynaptic origin, i.e. reduced released quanta of transmitter and increased synaptic delay. Only limited evidence suggests that reduced GABA release at HP in the CNS is caused by impaired Ca^{2+} entry into the presynaptic terminals. The extent at which HP

will affect a particular inhibitory synapse is probably similar to those associated with the AMPAR synapses (above).

Glycinergic synapses

Spinal cord synaptosomes experiments have demonstrated (Gilman et al., 1987; Gilman et al., 1989a) that HP consistently reduced and slowed the kinetics of glycine release. Indeed, HP completely abolished the very effective (54%) glycine dependent postsynaptic inhibition of the MSR and greatly reduced the recurrent inhibition. Furthermore, the test MSR response was even slightly facilitated by the conditioning pulse at the adjacent dorsal root (Schleifstein-Attias et al., 1994). Such a complete 'shut down' is unusual compared to other neurotransmitters that usually exhibit 40–60% depression of the synaptic response at 10.1 MPa HP. A possible explanation could be a difference in the postsynaptic receptors behavior at HP (see Chapter 7 in this book). Rat GlyRs expressed in frog oocytes show no change in the maximal response, but unlike the $GABA_AR$ did demonstrate a significant increase in the doze-response curve (EC50 increased by 60%) at 10.1 MPa (Shelton et al., 1993). Thus, postsynaptic reduction in receptor sensitivity will exacerbate HP suppression and would explain why the GlyR is more sensitive to pressure than the $GABA_AR$. A recombinant human GlyR has been also expressed in oocytes, exhibiting an even higher pressure sensitivity than the one in the rat (Roberts et al., 1996). Since glycine is an abundant inhibitory neurotransmitter in the spinal cord, it is of no surprise that one of the first HPNS symptoms are reduced motor coordination and muscle tremor.

In summary, the complete blockade of glycine transmission both by pre- and post synaptic mechanism is responsible for the sensitivity of the spinal cord to HP induced tremor and convulsions. It is conceivable that the large depression in AMPAR excitatory synaptic activity is causing functional difficulties for any neuronal circuit. However, it is the great depression of the inhibitory synaptic transmission in addition to increased activity of the NMDAR that brings about the hyperexcitability and epileptogenesis observed at HPNS.

MODULATORY SYNAPTIC ACTIVITY

Many of the CNS regulatory neurotransmitters are released by presynaptic mechanisms similar to those of the 'classical' excitatory and inhibitory neurotransmitters (as above), although the release could be slower. However, unlike the 'classical' neurotransmitters, they gate metabotropic receptors rather than ionotropic receptors. Thus, they do not directly change cellular ionic conductances or membrane potential. Instead, they bind to receptors

that are coupled through various G-proteins, which activate intracellular signaling cascades altering cell function. Early Pharmacological experiments on whole animals have indicated that pressure may affect monoamines receptors of dopamine (D1, D2) and serotonin (5-HT1b, 5-HT2c) (Abraini and Rostain, 1991; Abraini and Fechtali, 1992; Kriem et al., 1996, 1998). Further experiments suggest (see Chapter 20 in this volume, Bennett and Rostain, 2003) that HP may decrease the inhibitory actions of D2 receptors and stimulate the excitatory actions of D1 receptors. Furthermore, since both D1 and D2 receptors are predominantly expressed in the striatum and are known to be involved in motor control (e.g. Parkinson's disease, Gibb, 1992; Fearnley and Lees, 1991), these results do suggest that dopamine receptors may in part mediate the expression of acute HPNS.

Dopamine activity

As postulated, release of dopamine from synaptosomes preparations is depressed and slowed at HP (Gilman et al., 1988a; Gilman et al., 1989b). However, because of the great diversity of the signaling cascades, pressure effects are much more complicated. For example, D1 receptors stimulate adenylate cyclase (producing cAMP) and activate the cascade of protein kinase A. D2 receptors inhibit adenylate cyclase and separately activate arachidonic acid pathway (for details see Daniels and Grossman Chapter 7 in this volume). Direct evidence regarding dopamine synaptic activity at HP in the CNS is not available. However, in recent years a major effort was carried out in measuring *in vivo* dopamine (and serotonin) levels in the sub-cortical brain structures using voltametry (Rostain and Forni, 1995) or microdialysis (Darbin et al., 1997) methods. Exposure to HP increased the extracellular concentration of dopamine in rat and monkey striatum in association with increased locomotion and motor activity (for review see, Bennett and Rostain, 2003). Similar increase of dopamine has also been described for the nucleus accumbens, which is associated with emotional and mental states. The exact mechanism of increased extracellular concentration of dopamine is not clear. Direct effect on synaptic release is less likely but can not be ruled out. The possibility of higher synthesis without any change in degradation or uptake is suggested to occur at pressure (Bennett and Rostain, 2003). Some of these effects may result from changes in the network behavior rather than local changes. The general reduction of tonic network inhibition on the nigrostiatal and mesolimbic pathways, a reasonable outcome of the pressure induced depression of inhibitory processes (mentioned above), could facilitate release. As suggested above, a different role of D1 and D2 receptors is also postulated.

Serotonin activity

As postulated, release of serotonin (5-hidroxytriptamine, 5-HT) as well as norepinephrine from synaptosomes preparations is reduced and slowed under hyperbaric conditions (Gilman et al., 1988b; Gilman et al., 1989b). 5-HT1b receptors function through similar cascade as in D2 receptors. However 5-HT2C receptors activate phospholipase C, leading to the release of intracellular Ca^{2+} and protein kinase C activation. Further experiments in *Xenopus* oocytes (Daniels, unpublished data) suggested that pressure directly modulates the 5-HT2C receptors responses, whereas pressure effects on the 5-HT1b receptors appears to be mediated by up regulation of the cascade components.

In addition to the pharmacological studies, in recent years measurements of serotonin levels in the sub-cortical brain structures using voltametry and microdialysis were performed *in vivo* (as mentioned above for dopamine). Exposure to HP increased the extracellular concentration of serotonin in rat and monkey striatum and nucleus accumbens. It is suggested that pressure increases serotonin release by disinhibition of GABAergic post synaptic inhibition of the neurons via 5HT2A receptors. However, an opposite effect of reduction in release of serotonin may be mediated by another 5HT2C presynaptic autoreceptors (Bennett and Rostain, 2003).

In summary, dopaminergic and serotonergic systems are involved in pressure induced motor, locomotion, and possibly mental disorders (HPNS). The most prominent effect of pressure on the monoamines is elevation of their extracellular concentration in critical regions of the brain. However, the mechanism underlying this increase is unclear. Furthermore, this increase affects the receptors and their downstream signaling cascade in a very complicated and diverse mode. Thus, depending on the local pathways, pressure effects on any monoaminergic network could be differential, antagonistic, complementary, or even synergistic. The consequences of these changes are dependent on the network neurological task and the amount of control exerted by neighboring brain centers.

Acetylcholine activity

Cholinergic processes have a well established modulatory role in arousal and motor activity (Jones, 2004) as well as in various cognitive processes (Perry et al., 1999; Saar et al., 2007). However, centrally acting antagonists at muscarinic acetylcholine receptors (mAChR) (e.g. atropine) or nicotinic acetylcholine receptors (nAChR) (e.g. mecamylamine) do not affect the development of hyperexcitability at pressure (Wardley-Smith et al., 1984). The nAChR (abundant in the vertebrates neuromuscular junction) is not

sensitive to pressure (Heinemann et al., 1987b; for review see Chapter 7 in this book). However, studies in neuromuscular junction of frog (Ashford et al., 1982) and invertebrate central cholinergic synapses (Wann and Macdonald, 1980) indicate that HP slows the kinetics of the response and reduces spontaneous release of miniature EPSPs. Since the vertebrates (mammalian) motorneurons are cholinergic, acetylcholine is involved in various spinal inhibitory pathways that are activated by the motorneurons themselves. Pathways such as recurrent inhibition, Renshaw cells inhibition, and contralateral inhibition that involve activation of interneurons by the motorneurons are conceivably inhibited by HP partially because of reduced release of acetylcholine (Schleifstein-Attias and Grossman unpublished data). For similar reasoning depressed acetylcholine release may undermine impairment of cognitive functions at pressure.

SUMMARY

Presence of pressure sensitive molecules, especially ionic channels and receptors, in particular neurons and their localization in sub-cellular structures such as soma, axon or dendrites will all concert the response of each cell to pressure exposure. Furthermore on network levels, the incidence of affected neurons in the specific excitatory, inhibitory or modulatory network, the operating set-point of each synapse with regard to saturation that affect its frequency response, and the affected brain region(s) will all determine the final physiological response of the system to HP. More specifically, the mechanisms presently known to be involved in CNS response to HP exposure are summarized in Fig 1.

The less affected components of the system are the information lines of the presynaptic axons (Fig. 1, Input) and the postsynaptic axons (Fig. 1, Output). The action potentials amplitude and velocity are slightly reduced and their frequency response is not affected. However, the major characteristic of HPNS is the disruption of the delicate balance between excitation and inhibition. This could happen only if synaptic transmission (Fig. 1, Input component) and postsynaptic integration (Fig. 1, Integration) of the information are altered. Indeed, synaptic responses in both excitatory and inhibitory pathways (i.e. EPSPs and IPSPs) are suppressed at pressure. This general suppression is largely (but not exclusively) the consequence of reduced synaptic release due to impaired Ca^{2+} entry into the presynaptic terminals. If both excitatory and inhibitory release mechanisms are similarly suppressed quantitatively, imbalance between the two systems may occur only if additional mechanisms take place. Partial compensation for the reduced single synaptic response is attained by increased facilitation and in some cases by additional frequency potentiation. In contrast, in several synapses the normal twin pulse depression is unaffected and the frequency

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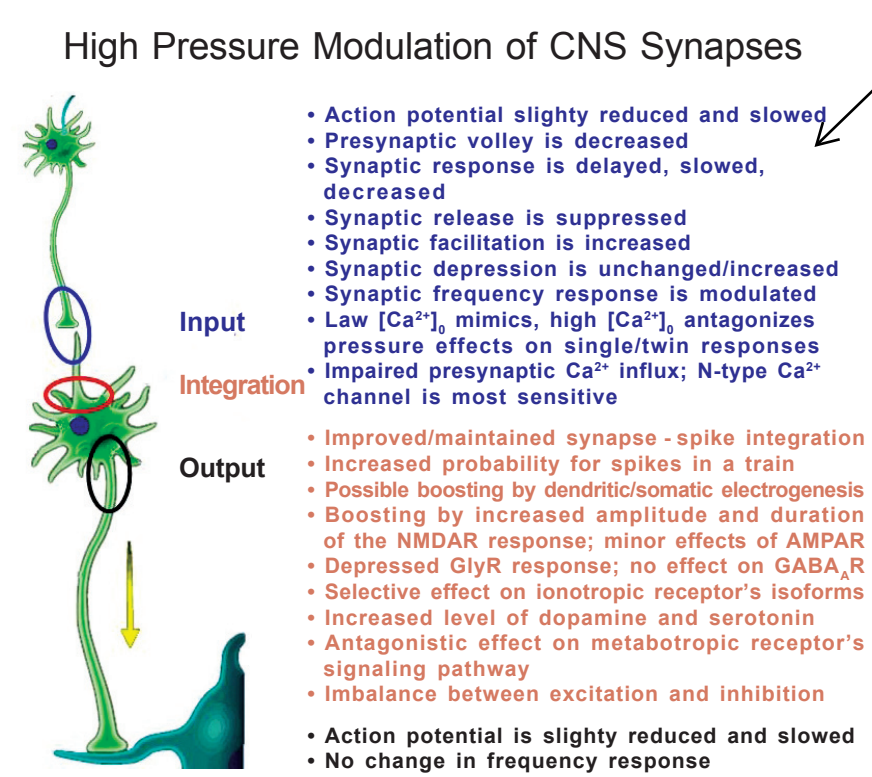

Figure 1. Summary of high pressure effects on synaptic transmission and network components.

depression is even increased. The role of synaptic frequency response in determining the type of 'behavioral' response is unclear. While the increase in synaptic efficacy in generating action potentials at 50 Hz may be related to seizure promotion, we may just speculate that the decline in the synaptic efficacy at < 25 Hz might correspond to minor reduction in motor performance (e.g. manual dexterity) or reduced cognitive capabilities. Another mechanism could be invoked due to differential pressure effect on the postsynaptic receptors. Boosting of excitatory response probably occurs when NMDAR (but not AMPAR) responses are increased, while strong disinhibition may be effected when the GlyR (but not $GABA_AR$) response is practically shut down. It is important to note that pressure effects may be selective for various isoforms of the receptors. Another mechanism that may bring about hyperexcitability could be additional activation of voltage dependent channels (such as specific Ca^{2+} channels) along the dendrites or soma of the neurons. This may boost the reduced synaptic potentials and increase their efficacy in generating action potentials. Again, it is worth

noting that pressure effects may be selective for various types of ionic channels. Finally, the excitatory and inhibitory systems are modulated by metabotropic receptors of monoamines such as serotonin and dopamine, while the monoaminergic neurons themselves can be inhibited by feedback circuits. Here the major effect of pressure is the increase of monoamines extracellular concentrations in the vicinity of their target neurons. However, this rise differentially affects various neurons (either in motor or cognitive system) depending on the type of receptor and its specific signaling cascade.

CONCLUSION

To understand how pressure affects the CNS is an enormous scientific challenge. Many of the CNS functions and mechanisms are highly complex and remain a mystery for modern science. Apparently the task could be somewhat easier, since unlike a chemical, pressure acts 'instantaneously' and throughout the brain. This may mean that pharmacokinetics and metabolism are not confounding problems. However, this also indicates that the scope of research is wide open since, in principle, every molecule (protein) and any chemical reaction is a potential target. Furthermore, even when a family of a receptor, for example, is identified as pressure sensitive, pressure effects could be selective or differential for various isoforms of the receptor.

The common notion of short term pressure effects on the CNS are summarized above. Yet, our recent finding of the possibility of excessive Ca^{2+} influx into the neurons during increased activation of the NMDAR suggests that HP may exert some long term effect on the cells' viability (neurotoxicity). Although the effect of single exposure can be minor, the long term effect following repetitive exposures during many years of deep diving may be significant.

There are many aspects of HPNS that are far less understood. Given the range of targets for modification by pressure and the complexity of the CNS, a lot remains to be elucidated before a comprehensive understanding of the effect of pressure on CNS function could be claimed.

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